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Ginseng and ginsenoside Rg_3, a newly identified active ingredient of ginseng, modulate Ca^{2+} channel currents in rat sensory neurons

Hyewhon Rhim [a,*], Hyeno Kim [a], Dong Yoon Lee [a], Tae Hwan Oh [b], Seung-Yeol Nah [c]

[a] *Biomedical Research Center, Korea Institute of Science and Technology, 39-1 Hawholgok-dong Sungbuk-gu, Seoul 136-791, South Korea*
[b] *Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[c] *Department of Physiology, College of Veterinary Medicine, Chonnam National University, Kwangju 500-757, South Korea*

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Abstract

There is increasing evidence that ginseng influences pain modulation. In spite of extensive behavior studies, the detailed mechanism of ginseng actions at the cellular level and the identity of the active substance have not been elucidated yet. Whole-cell patch-clamp recordings were used to examine the modulation of high-voltage-activated Ca^{2+} channel currents by ginseng total saponins and its various individual ginsenosides in rat dorsal root ganglion neurons. Application of ginseng total saponins suppressed Ca^{2+} channel currents in a dose-dependent manner. Occlusion experiments using selective blockers revealed that ginseng total saponins could modulate L-, N-, and P-type currents. The co-application of ginseng total saponins and the μ-opioid receptor agonist, D-Ala^2, *N*-MePhe^4, Gly^5-ol-enkephalin (DAMGO), produced non-additive effects in most cells tested and each effect was significantly relieved by a depolarizing prepulse. Overnight treatment of cells with pertussis toxin profoundly reduced the inhibition. Furthermore, we now report that ginsenoside Rg_3, among the major fractions of ginseng saponins, is a newly identified active component for the inhibition. These results suggest that the modulation of Ca^{2+} channels by ginseng total saponins, in particular by ginsenoside Rg_3, could be part of the pharmacological basis of ginseng-mediated antinociception.

Keywords: Ginseng; Ginsenoside; Antinociception; Patch-clamp; Ca^{2+} channel current; Pertussis toxin; Sensory neurons

1. Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer (*Araliaceae*), has been used worldwide as an herbal medicine. Ginsenosides or ginseng saponins are the main molecular components responsible for the actions of ginseng and more than 30 types of ginsenosides have been identified (Liu and Xiao, 1992; Baek et al., 1996). Among the efficacies of ginseng which produces an array of pharmacological responses (Liu and Xiao, 1992; Attele et al., 1999), several studies have shown antinociceptive actions of ginseng extracts in various behavior assays (Kim et al., 1990; Ramarao and Bhargava, 1990; Bhargava and Ramarao, 1991; Choi et al., 2000). For example, systemic injections of ginseng extracts produced antinociception on acute treatment and inhibited the development of tolerance to the analgesic effects of morphine (Kim et al., 1990; Ramarao and Bhargava, 1990; Bhargava and Ramarao, 1991). A recent study also showed that i.t. administration of ginsenosides produced antinociception and prevented the opioid tolerance caused by chronic treatment with morphine at spinal sites (Choi et al., 2000). These studies indicate that ginseng plays an important role in modulating pain perception in the peripheral nervous system as well as the central nervous system. However, compared to the extensive studies in animal experiments, the identification of the active substance and its cellular mechanism of action have not been fully elucidated yet.

Since results of previous studies suggested that ginseng mediates pain perception by mimicking the effects of opioids (Nah and McCleskey, 1994; Choi et al., 2000), it is important to examine the effects of ginseng on high-voltage-activated Ca^{2+} channels which are involved in opioid-mediated pain perception by reducing Ca^{2+} entry (Moises et al., 1994; Nomura et al., 1994; Rhim and Miller, 1994; Rhim et al., 1996) and to compare the signal pathways of ginseng and opioid receptors at the single-cell level. Nah et al. (1995) reported that a trace component of ginseng, ginsenoside Rf, inhibited Ca^{2+} channels on primary sensory neurons and is

* Corresponding author. Tel.: +82-2-958-5923; fax: +82-2-958-5909.
E-mail address: hrhim@kist.re.kr (H. Rhim).

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the active ingredient in ginseng that mimics opioids. However, differences in the nature of the antinociception with ginsenoside Rf (Mogil et al., 1998) and that reported for crude ginseng extracts (Ramarao and Bhargava, 1990; Bhargava and Ramarao, 1991) using behavior assays suggest that ginsenoside Rf is not the only active compound in the extracts.

We, therefore, investigated the effects of ginseng total saponins on the modulation of high-voltage-activated Ca^{2+} channel currents using the whole-cell patch-clamp technique with acutely isolated rat dorsal root ganglion neurons and also performed some parallel experiments using a μ-opioid receptor agonist to compare signal pathways between two systems. We further identified the active fraction of ginseng saponins and characterized ginsenoside Rg_3 as a potent inhibitor of Ca^{2+} channels in dorsal root ganglion neurons.

2. Materials and methods

2.1. Materials

Ginseng total saponins and various individual ginsenosides were obtained from the Korea Ginseng and Tobacco Research Institute (Taejon, Korea). The percentage of various ginsenosides in ginseng total saponins were as follows: ginsenoside Ra (2.91%), ginsenoside Rb_1 (18.26%), ginsenoside Rb_2 (9.07%), ginsenoside Rc (9.65%), ginsenoside Rd (8.24%), ginsenoside Re (9.28%), ginsenoside Rf (3.48%), ginsenoside Rg_1 (6.42%), ginsenoside Rg_2 (3.63%), ginsenoside Rg_3 (4.70%), ginsenoside Ro (3.82%), and other minor components. Ginseng total saponins were dissolved in external recording solution and individual ginsenosides were dissolved in methanol or dimethylsulfoxide (DMSO) as a concentrated stock and further diluted to its final concentration in external recording solution. We found the final concentration of methanol or DMSO ($<0.1\%$) had no effect on Ba^{2+} currents, but as a precaution, the external recording solution in initial experiments contained matched methanol or DMSO concentrations when they were used at 0.1% concentration. Nifedipine, ω-conotoxin GVIA and ω-agatoxin IVA were purchased from Calbiochem (San Diego, CA), Alomone Labs (Jerusalem, Israel) and Peptide Institute (Osaka, Japan), respectively. All other chemicals were purchased from Sigma (St. Louis, MO). Drugs were applied in the perfusate by gravity. All experiments were performed at room temperature.

2.2. Cell preparation

Sprague-Dawley rats (100-150 g) were anesthetized with ether by inhalation and killed by decapitation. Dorsal root ganglia were dissected and incubated with 0.15% collagenase and then with 0.125% trypsin in Ca^{2+}- and Mg^{2+}-free HEPES buffer solution at 37 °C. Dorsal root ganglion neurons were then mechanically dissociated with fire-polished Pasteur pipettes by trituration and plated on poly-L-lysine-coated coverslips in a 35-mm culture dish. Cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin under a humidified atmosphere of 95% air/5% CO_2 at 37 °C. For electrophysiological recordings, the cells were used within 1 day after plating. All chemicals for cell preparation were purchased from Gibco (Grand Island, NY). For pertussis toxin experiments, the cells were treated with toxin for 18–24 h at 37 °C before recordings were made. Pertussis toxin was stored as a 100 μg/ml solution in purified water and diluted into cultures of isolated neurons to a final concentration of 200 or 500 ng/ml. As controls, cells received the same amount of culture medium instead of toxin during a similar incubation period.

2.3. Electrophysiology

Whole-cell voltage-clamp recordings were used (Hamill et al., 1981). Glass electrodes with resistance of 2–3 MΩ were pulled using a Narishige two-stage vertical puller and coated with Sylgard (Dow Corning, Midland, MI). High-voltage activated Ba^{2+} currents were recorded using Ba^{2+} as the charge carrier, and Cs^+ and tetraethylammonium as the predominant internal and external cations, respectively, and evoked every 15 s by a 200-ms depolarizing voltage step from −80 to −20 mV. The internal recording solution consisted of (in mM): 120 CsCl, 10 HEPES, 2 $MgCl_2$, 10 EGTA, 5 ATP-Mg, 0.3 GTP-Tris, 14 Tris-creatine phosphate, and 50 U/ml creatine phosphokinase, pH-adjusted to 7.4 with CsOH. The external solution consisted of (in mM): 150 tetraethylammonium chloride, 2 $BaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, pH-adjusted to 7.4 with tetraethylammonium hydroxide. Current recordings were obtained with an EPC-9 amplifier and Pulse/Pulsefit software (HEKA, Germany). The series resistance was compensated up to 75–90%.

2.4. Data analysis

Percentage inhibition was calculated as $100[(C-D)/C]$, where D is the peak current in the presence of ginseng total saponins, and C is the peak current before the application of ginseng total saponins. In some cells, a corrected C value at the time of maximal response to drug was obtained from a line between control and recovery currents and was used for calculations of percentage inhibition due to taking run-down into account. All data were expressed as means ± S.E.M. When data are plotted as bar graphs, the number above the bar indicates the number of cells tested. Statistical significance was determined by using Student's t-test and was set at the $P<0.05$ level. To determine the IC_{50} value of the effect of ginseng total saponins on Ba^{2+} currents, we used the four-parameter logistic equation

$$I = I_{\min} + (I_{\max} - I_{\min})/(1 + 10^{(\log IC_{50} - X)\cdot n}) \quad (1)$$

where I denotes inhibition of the current in the presence of ginseng total saponins, I_{min} and I_{max} are the minimum and maximum inhibition of Ba^{2+} currents by ginseng total saponins, respectively, X is the logarithm of ginseng total saponins concentration, and n is the Hill coefficient.

3. Results

Fig. 1A illustrates the effect of ginseng total saponins on peak high-voltage-activated Ba^{2+} currents in one acutely isolated dorsal root ganglion neuron. The application of ginseng total saponins (100 μg/ml) produced 28.5% inhibition of the peak Ba^{2+} currents ($I_{Ba^{2+}}$) that returned to control levels after washout of the drug and was again produced by a second exposure. The mean percentage inhibition by ginseng total saponins (100 μg/ml) was 27.8 ± 1.6% from 46 cells. This ginseng total saponins-mediated inhibition of $I_{Ba^{2+}}$ occurred in a dose-dependent manner (Fig. 1B). When the data in Fig. 1B were fitted by computer to Eq. (1) (see Materials and methods for details), the concentration of ginseng total saponins producing half-maximal inhibition (IC_{50}) of the current was calculated to be 61.4 μg/ml with a Hill coefficient of 2.65. To determine which types of high-voltage-activated Ca^{2+} channels were being inhibited by ginseng total saponins, occlusion experiments with selective Ca^{2+} channel blockers were performed. The suppression of $I_{Ba^{2+}}$ by ginseng total saponins was measured before and after application of each blocker. The mean reduction of $I_{Ba^{2+}}$ by the L-type channel blocker, nifedipine (10 μM), the N-type blocker, ω-conotoxin-GVIA (ω-CgTx-GVIA, 1 μM), and the P-type blocker, ω-agatoxin-IVA (100 nM), was 18.5 ± 2.9%, 47.6 ± 6.2%, and 18.7 ± 6.4% (n = 5), respectively, which agreed very well with published data for these cells (Mintz et al., 1992; Acosta and Lopez, 1999). Fig. 1C illustrates the effect of ginseng total saponins before and after application of Ca^{2+} channel blockers. Ginseng total saponins suppressed the 38.0 ± 3.3% inhibition of peak current before and 14.1 ± 3.4% after nifedipine (10 μM) block in five cells. Treatment with the N-type blocker, ω-CgTx-GVIA (1 μM), also decreased the inhibition from 37.1 ± 6.4% to 16.3 ± 4.1% in five cells. On the other hand, the P-type channel toxin, ω-agatoxin-IVA (100 nM), only partially blocked the effect of ginseng total saponins (24.0 ± 3.0% before and 19.7 ± 2.3% after toxin treatment, n = 5). These results suggest that ginseng total saponins can modulate L-, N-, and P-types of Ba^{2+} currents in rat dorsal root ganglion neurons.

Since ginseng has been proposed to mimic the effects of opioids in mediating pain perception, we performed some parallel experiments using D-Ala^2, *N*-MePhe^4, Gly^5-ol-enkephalin (DAMGO), the μ-opioid receptor agonist, to compare signal pathways of ginseng and opioid receptors (Fig. 2). First, we examined the effects of simultaneous exposure to a pair of agonists in a single neuron. After confirming the responsiveness of a cell to each agonist, co-

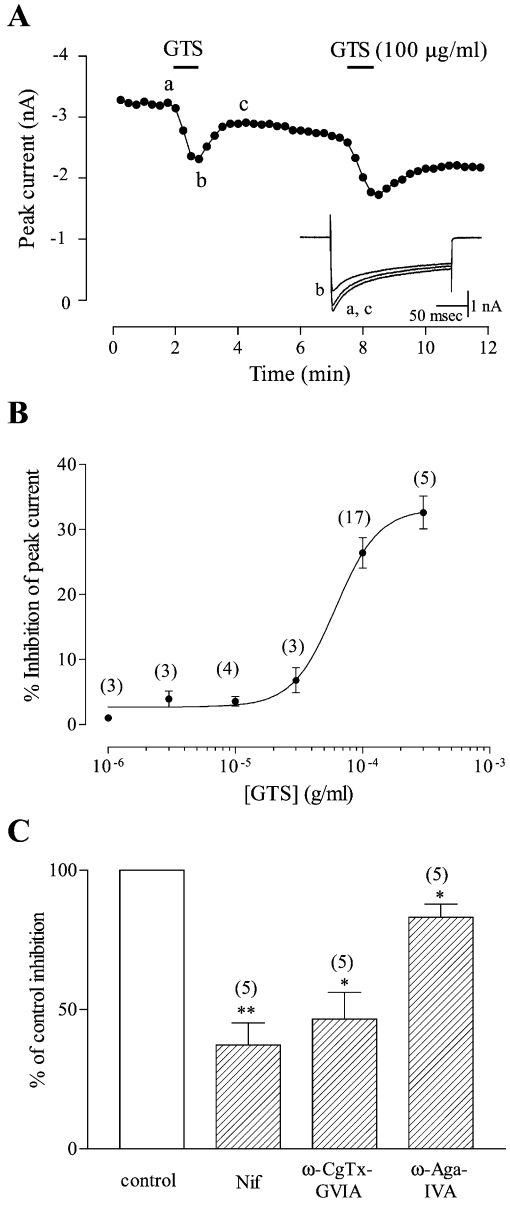


Fig. 1. Ginseng total saponins inhibited high-voltage-activated Ba^{2+} currents in rat dorsal root ganglion neurons. (A) Time course of peak Ba^{2+} current ($I_{Ba^{2+}}$) showing two exposures to 100 μg/ml ginseng total saponins (GTS). Inset, Leak-subtracted currents at labeled points from the time course of peak currents. Ba^{2+} currents were evoked every 15 s by a 200-ms voltage step from − 80 to − 20 mV using Ba^{2+} (2 mM) as the charge carrier. (B) Dose–response relationship for inhibition of $I_{Ba^{2+}}$ by ginseng total saponins. The solid line connecting the points represents the best computer fit of the data to Eq. (1). The IC_{50} of the effect of ginseng total saponins on $I_{Ba^{2+}}$ is 61.4 μg/ml and the Hill coefficient (n) is 2.65. The cells treated at a low concentration were subsequently tested at a high concentration to check the responsiveness of a cell to ginseng total saponins. Numbers above each point indicate the number of cells tested at each concentration. (C) Pooled results are shown from Ca^{2+} channel blocker experiments illustrating the percentage of ginseng total saponins-mediated modulation in the presence of each channel blocker. The suppression of $I_{Ba^{2+}}$ by ginseng total saponins before the application of toxin was calculated as 100% control for each cell tested. Nifedipine (Nif, 10 μM), ω-conotoxin-GVIA (ω-CgTx-GVIA, 1 μM), and ω-agatoxin-IVA (ω-Aga-IVA, 100 nM) occluded about 62.7%, 53.4%, and 16.9% of the effect of ginseng total saponins, respectively ($*P < 0.05$ and $**P < 0.01$, compared with each control value before the application of toxin).

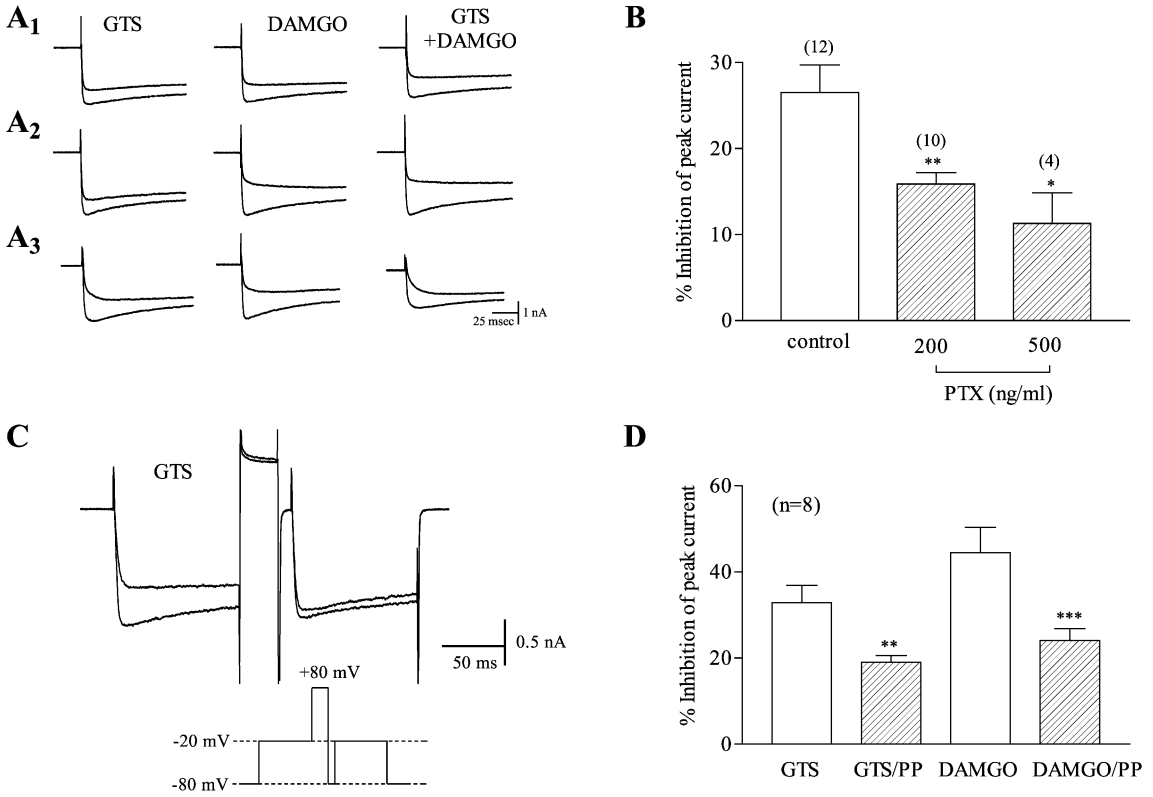
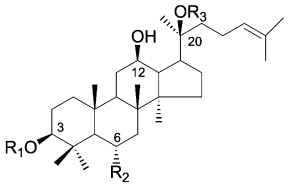


Fig. 2. Comparison of ginseng total saponins- and DAMGO-mediated inhibition of high-voltage-activated Ba^{2+} currents. When ginseng total saponins (100 μg/ml) and the μ-opioid agonist, DAMGO (1 μM), were applied simultaneously to a single neuron after the responsiveness of a cell to each agonist was confirmed, they did not produce any additive inhibition on $I_{Ba^{2+}}$ in 11 cells of 13 tested. (A_1-A_3) Examples of cells showing effects in a non-additive manner. (B) Effects of pertussis toxin (PTX) treatment on ginseng total saponins-mediated inhibition of $I_{Ba^{2+}}$. Experiments were conducted alternately on control cells and on cells treated with pertussis toxin (200 or 500 ng/ml). Cells were treated with toxin for 18 to 24 h at 37 °C before recordings were made. $*P<0.05$ and $**P<0.01$, compared with control cells. (C) A strong depolarizing prepulse (+80 mV for 30 ms, PP) was applied at the end of the first voltage step (from −80 to −20 mV for 100 ms) with a 10-ms interval to the second one as shown in the diagram. The overlapped current traces obtained from the control and in the presence of ginseng total saponins. The depolarizing prepulse accelerated ginseng total saponins-induced slowing of the activation of the current and relieved the inhibition of $I_{Ba^{2+}}$, which was similar to the effects of opioids (Nomura et al., 1994; Rhim et al., 1996). (D) Pooled results from cells tested in prepulse protocols. In the presence of drug, open bars represented the inhibition of peak $I_{Ba^{2+}}$ obtained from the first voltage step and hatched bars, from the second one following a strong prepulse (PP). The significant relief of inhibition by a prepulse was observed for both ginseng total saponins- and DAMGO-mediated effects. $**P<0.01$ and $***P<0.001$, compared with each control value.

application of ginseng total saponins (100 μg/ml) and DAMGO (1 μM) did not produce any additive inhibition in 11 cells among 13 tested. Additivity on the inhibition of $I_{Ba^{2+}}$ was not observed, even in cells showing either moderate (Fig. 2A_1 and A_2) or large (Fig. 2A_3) inhibition by ginseng total saponins. Secondly, we examined the effect of pertussis toxin and voltage-dependence of inhibition on ginseng total saponins-mediated modulation as similar pathways for the inhibition of the Ca^{2+} channel by activation of μ-opioid receptors (Moises et al., 1994; Nomura et al., 1994; Rhim et al., 1996). Experiments were conducted alternately on control cells and on cells treated with pertussis toxin. Inhibition of $I_{Ba^{2+}}$ mediated by ginseng total saponins was greatly reduced by treatment with pertussis toxin (Fig. 2B). In control cells, the mean percentage inhibition by ginseng total saponins was $26.5 \pm 3.2\%$ ($n=12$). However, in cells incubated with 200 and 500 ng/ml pertussis toxin for 18–24 h at 37 °C, ginseng total saponins reduced $I_{Ba^{2+}}$ by $15.9 \pm 1.3\%$ ($n=10$) and $11.3 \pm 3.6\%$ ($n=4$), respectively. Fig. 2C shows that the ginseng total saponins-mediated inhibition of $I_{Ba^{2+}}$ was partially relieved by a strong depolarizing prepulse (+80 mV for 30 ms) similar to the partial relief of DAMGO-mediated inhibition of the Ca^{2+} channel by a similar prepulse protocol (Nomura et al., 1994; Rhim et al., 1996). Fig. 2D compares the effects of a strong depolarizing prepulse on ginseng total saponins- and DAMGO-mediated inhibition of $I_{Ba^{2+}}$ from eight cells tested. The mean inhibition of $I_{Ba^{2+}}$ produced by ginseng total saponins was $32.9 \pm 4.0\%$ before a prepulse and this was decreased to $19.0 \pm 1.5\%$ following a depolarizing prepulse ($**P<0.01$). In the same way, the mean inhibition of $I_{Ba^{2+}}$ by DAMGO was $44.5 \pm 5.9\%$ before a prepulse and this was decreased to $24.1 \pm 2.8\%$ by a depolarizing prepulse ($***P<0.001$). These results sug-

gest that ginseng total saponins and μ-opioid receptors can share some signal pathways in the modulation of Ba^{2+} currents in dorsal root ganglion neurons.

Previous studies suggest that ginsenoside Rf is not the only active compound in crude ginseng extracts because there were differences in the nature of the antinociception with ginsenoside Rf and that reported for the extracts, using behavior assays (Ramarao and Bhargava, 1990; Bhargava and Ramarao, 1991; Mogil et al., 1998). Therefore, we examined the inhibitory effects of 11 ginsenosides, major individual components of ginseng, on high-voltage-activated Ca^{2+} channels, in comparison with the effect of ginsenoside Rf which was previously reported as an active component responsible for inhibiting Ca^{2+} channels in sensory neurons (Nah et al., 1995; Mogil et al., 1998). Fig. 3 shows the chemical structures of the ginsenosides now tested and their classification into panaxadiol or panaxatriol saponins according to the characteristics of their chemical structures: they differ as to sugar moiety at carbon-3, -6, and -20 (Shibata et al., 1966; Tanaka et al., 1966). Among the 11 ginsenosides tested at 100 μM, ginsenoside Rg_3 was found to be the most potent fraction of ginseng for the inhibition of $I_{Ba^{2+}}$ (Fig. 4). The mean percentage inhibition by the panaxadiol saponin group (Fig. 4A) was as follows: ginsenoside Rb_1 (12.0 ± 2.4%, $n=4$), ginsenoside Rb_2 (13.0 ± 2.1%, $n=3$), ginsenoside Rc (22.8 ± 4.9%, $n=7$), ginsenoside Rd (11.2 ± 3.9%, $n=3$), ginsenoside Rg_3 (47.8.0 ± 6.0%, $n=10$), ginsenoside Rh_2 (19.0 ± 3.6%, $n=8$). The



Ginsenosides	R_1	R_2	R_3	PD or PT
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc	PD
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)	PD
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)	PD
Rd	-Glc_2-Glc	-H	-Glc	PD
Re	-H	-O-Glc_2-Rha	-Glc	PT
Rf	-H	-O-Glc_2-Glc	-H	PT
Rg_1	-H	-O-Glc	-Glc	PT
Rg_2	-H	-O-Glc_2-Rha	-H	PT
Rg_3	-Glc_2-Glc	-H	-H	PD
Rh_1	-H	-O-Glc_2	-H	PT
Rh_2	-Glc_2	-H	-H	PD

Fig. 3. Chemical structures of the eleven ginsenosides studied on high-voltage-activated Ba^{2+} currents. Ginsenosides differ at side-chains attached to the common steroid rings. They are also further classified into panaxadiol (PD) and panaxatriol (PT) saponins according to the characteristics of their chemical structures (Shibata et al., 1966; Tanaka et al., 1966). Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (fur), arabinofuranose; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Numbers indicate the carbon in the glucose ring that links the two carbohydrates.

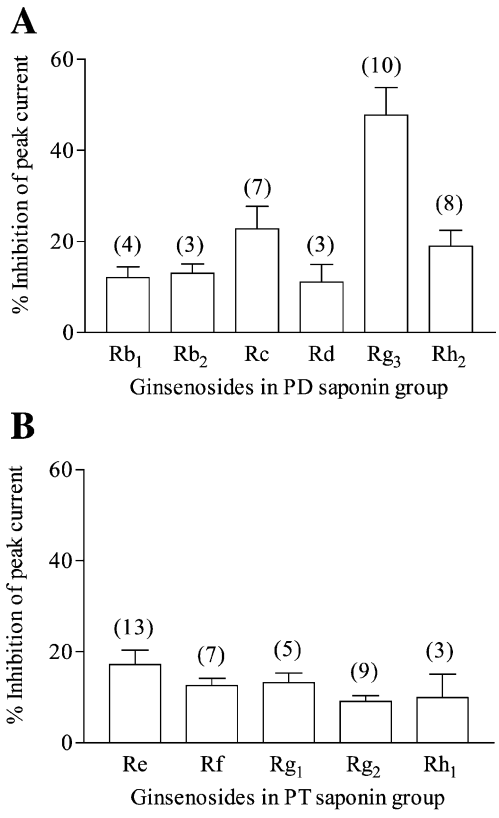


Fig. 4. Effects of individual ginsenosides on high-voltage-activated Ba^{2+} currents. Pooled results illustrating the mean percentage inhibition of peak currents by ginsenosides in the panaxadiol (PD, A) and panaxatriol (PT, B) saponin groups. Three ginsenosides showing the largest degree of inhibition, ginsenosides Rg_3, Rc, Rh_2, produced the effect in all cells tested and they are all belonged to the panaxadiol saponin group.

mean percentage inhibition by the panaxatriol saponin group (Fig. 4B) was as follows: ginsenoside Re (17.2 ± 3.2%, $n=13$), ginsenoside Rf (12.6 ± 1.6%, $n=7$), ginsenoside Rg_1 (13.2 ± 2.2%, $n=5$), ginsenoside Rg_2 (9.1 ± 1.3%, $n=9$), ginsenoside Rh_1 (9.9 ± 5.1%, $n=3$). Especially because the previous studies showed that ginsenoside Rf was the main component responsible for inhibiting Ca^{2+} channels in sensory neurons (Nah et al., 1995; Mogil et al., 1998), we compared the effects of ginsenosides Rf and Rg_3 in the same cell. Application of ginsenoside Rg_3 caused 65.4% inhibition of $I_{Ba^{2+}}$ while ginsenosides Rf and Re produced 14.8% and 5.9% inhibition at 100 μM, respectively, as shown in Fig. 5A. From the pooled data for four cells, the mean percentage inhibition of $I_{Ba^{2+}}$ by ginsenosides Rg_3 and Rf was 49.2 ± 10.5% and 15.9 ± 2.0%, respectively. The ginsenoside Rg_3-mediated inhibition also was dose-dependent and a similar extent of inhibition was observed with a purified stereoisomer of ginsenoside Rg_3, 20 (*S*)-ginsenoside Rg_3, as shown in Fig. 5B. When cells were pretreated with 200 ng/ml pertussis toxin for 18–24 h at 37 °C before recordings were made, the inhibition of $I_{Ba^{2+}}$

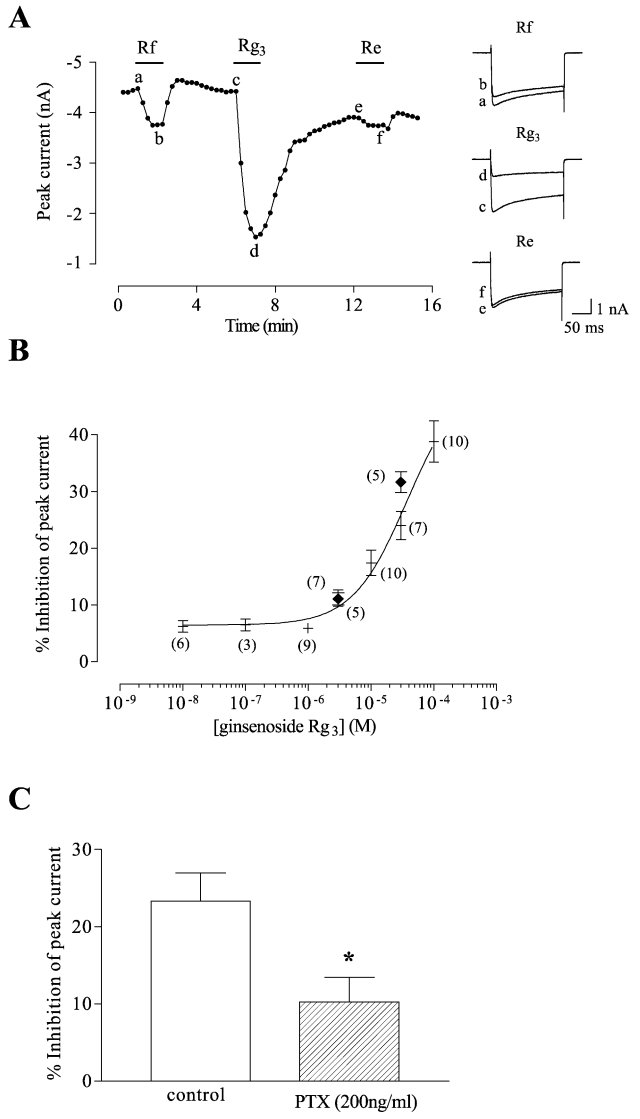


Fig. 5. Effects of ginsenoside Rg_3 on high-voltage-activated Ba^{2+} currents. (A) Left panel, time course of peak currents illustrating effects of different ginsenosides Rf, Rg_3 and Re in one neuron. At 100 μM concentration, ginsenosides Rf (Rf) and Re (Re) inhibited currents by 14.8% and 5.9%, respectively, while ginsenoside Rg_3 (Rg_3) produced the largest inhibition (65.4%) in this cell. Right panel, leak-subtracted currents at labeled points of the time course of peak currents. (B) Dose–response relationship for inhibition of $I_{Ba^{2+}}$ by ginsenoside Rg_3 (symbol +). This solid line connecting the points also represents the best computer fit of the data to Eq. (1). A purified stereoisomer of ginsenoside Rg_3, 20 (*S*)-ginsenoside Rg_3 (symbol ♦), produced similar inhibition to that with ginsenoside Rg_3. (C) Pertussis toxin-sensitive G-protein-mediated effects of ginsenoside Rg_3. Ginsenoside Rg_3 (30 μM) reduced $I_{Ba^{2+}}$ by $10.3 \pm 3.2\%$ ($n=4$) in cells treated with pertussis toxin (200 ng/ml, $23.3 \pm 3.7\%$ for four control cells incubated in culture medium without toxin). $^*P<0.05$ with control cells.

by ginsenoside Rg_3 (30 μM) was greatly reduced (Fig. 5C). Ginsenoside Rg_3 at 30 μM reduced $I_{Ba^{2+}}$ by $10.3 \pm 3.2\%$ ($n=4$) in cells treated with pertussis toxin ($23.3 \pm 3.7\%$ for four control cells incubated in culture medium without toxin). These results suggest that ginsenoside Rg_3 is one of the main active components responsible for ginseng total saponins-mediated inhibition of Ca^{2+} channel currents via pertussis toxin-sensitive G-protein-mediated pathways.

4. Discussion

In the present study, we characterized the modulation by ginseng total saponins of Ba^{2+} currents and further identified the active components responsible for ginseng total saponins-mediated inhibition in rat dorsal root ganglion neurons. Our results show that ginseng total saponins inhibited L-, N-, and P-type high-voltage-activated Ba^{2+} currents via pertussis toxin-sensitive G-protein pathways and ginsenoside Rg_3 is one of the main active components responsible for ginseng actions.

Application of ginseng total saponins produced a rapid inhibition of Ba^{2+} currents (Fig. 1) and slowed the activation of Ba^{2+} currents during a test step pulse in some cells (Fig. 2A and C). Since the inhibitory effect of G-protein-mediated actions on the Ca^{2+} channel is known to be slowing of the activation of Ba^{2+} currents, and since relief of the inhibition is accelerated by strong positive membrane potentials, we examined the involvement of G-proteins (probably G_i/G_o), using a pertussis toxin and a depolarizing prepulse protocol. The inhibition of Ba^{2+} currents by ginseng total saponins was reduced by treatment with pertussis toxin (Fig. 2B) and partially relieved by application of a strong depolarizing prepulse (Fig. 2C and D). This suggests that a pertussis toxin-sensitive G-protein was involved in ginseng total saponins-mediated inhibition through a mechanism similar to that proposed for G-protein-mediated neurotransmitters, including opioid receptors (Moises et al., 1994; Nomura et al., 1994; Rhim et al., 1996). The relief of inhibition by a prepulse could be interpreted as a voltage-dependent transition between "reluctant" and "willing" modes of Ca^{2+} channels mediated by G-proteins (Bean, 1989). However, results of a recent study also suggested that this relief action could be regulated by protein kinase C (Herlitze et al., 2001), which we did not test in this study.

The result of simultaneous exposures to ginseng total saponins and DAMGO in single neurons (Fig. 2A) also suggests that ginseng total saponins act on sensory neurons through a pathway similar to that for μ-opioid receptors. However, this is unlikely to occur at the level of opioid receptors, since naloxone, an antagonist of opioid receptors, did not block the inhibitory effect of ginseng extracts on Ca^{2+} channels (Nah and McCleskey, 1994). Taken together, our findings suggest that ginseng total saponins could share some signal pathways with opioid receptors, particularly at the level of pertussis toxin-sensitive G-proteins or N- or P-type Ca^{2+} channels which are well known as important signal molecules in opioid actions (Moises et al., 1994; Nomura et al., 1994; Rhim and Miller, 1994; Rhim et al., 1996).

These could potentially serve to explain the mechanisms of ginseng-mediated actions; both its own analgesic effects and blockade of opioid tolerance. For ginseng's own analgesic effects, it is possible that ginseng produces antinociception by inhibiting Ca^{2+}-evoked neurotransmitter release through Ca^{2+} channels as do opioids. In addition, we also speculate about one of possibilities for the mechanism of blockade of opioid tolerance by ginseng. Since ginseng total saponins and opioids share some pathways in the modulation of Ca^{2+} channels, it is conceivable that ginseng could use common signal molecules such as G-proteins or N- or P-type Ca^{2+} channels which may be required for the full activation of opioid receptors. Therefore, pre- or co-activation of ginseng total saponins via pertussis toxin-sensitive G-proteins or Ca^{2+} channels might decrease the efficacy of opioid actions and apparently suppress the development of tolerance to opioid antinociception.

However, there is another possibility, that ginseng might act via pathways other than the opioid system in several aspects. These points are: (i) ginseng total saponins inhibited L-type Ca^{2+} channels that were not modulated by μ-opioid (Moises et al., 1994; Rhim and Miller, 1994); (ii) the co-application of ginseng and DAMGO produced additive effects in a subpopulation of dorsal root ganglion neurons (2 of 13 cells tested); (iii) ginseng saponins attenuate mainly chemical-induced pain but not noxious heat-induced pain in in vivo experiments (Yoon et al., 1998).

For the application of natural compounds as a potential therapeutic choice, it is necessary to identify an active component that is responsible for the effect. Up to date, ginsenoside Rf has been known as the active component reproducing the inhibitory effect of ginseng extracts on Ca^{2+} channels in sensory neurons (Nah et al., 1995). However, behavior studies showed that ginsenoside Rf inhibited only tonic pain without affecting nociception measured in acute pain assays while ginseng extract or ginseng total saponins affected both types of pain (Ramarao and Bhargava, 1990; Bhargava and Ramarao, 1991; Mogil et al., 1998). These differences in the nature of the antinociception between the extracts and ginsenoside Rf suggested that it is highly unlikely that ginsenoside Rf is the only active compound in the extracts. In the present study, we showed that ginsenoside Rg_3 was the most potent fraction of ginseng saponins and a more marked inhibitor of $I_{Ba^{2+}}$ than is ginsenoside Rf (Fig. 5). Furthermore, this ginsenoside Rg_3-mediated inhibition also occurred via pertussis toxin-sensitive G-protein-mediated pathways as do ginseng total saponins. Although we examined most main components of ginseng saponins and identified ginsenoside Rg_3 as the active component, further behavior assay of ginsenoside Rg_3 would be valuable for identifying the active compound in ginseng-mediated antinociception.

Based on results from a number of different assay systems, it has been reported that ginsenoside Rg_3 seems to be a very interesting and important component of ginseng. For example, ginsenoside Rg_3 inhibited cell growth in human prostate carcinoma cells (Liu et al., 2000) and vascular smooth muscle tone via activation of K^+ channels (Kim et al., 1999). For the modulation of receptors, ginsenoside Rg_3 was described as not only a blocker of ionotropic receptors but also an antagonist of muscarinic or histamine receptors in bovine adrenal chromaffin cells (Tachikawa et al., 1999). Our finding of ginsenoside Rg_3 as a new active component is well consistent with results of the previous study which examined the effects of ginsenosides on neuroprotection in cultured cortical cells (Kim et al., 1998). They found ginsenoside Rg_3 to be one of the major active components for inhibiting glutamate-induced neuronal cell death and Ca^{2+} influx through glutamate receptors. Taken together, although ginsenoside Rg_3 is present in only trace amount within ginseng, it could be one of pharmacological bases of ginseng-mediated actions either by itself or along with other characterized components such as ginsenoside Rf.

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